Volume 219, number 2, 347–350 FEB 04920 July 1987

Na^+-induced uptake of pyruvate into mesophyll chloroplasts of a C_4 plant, *Panicum miliaceum*

Jun-Ichi Ohnishi and Ryuzi Kanai

Department of Biochemistry, Faculty of Science, Saitama University, Urawa 338, Japan

Received 28 April 1987

Uptake of [1-^{14}C]pyruvate into the sorbitol-impermeable space of mesophyll chloroplasts of a C_4 plant, *Panicum miliaceum* L., was investigated using a silicone oil filtering centrifugation method. An abrupt change of Na^+ concentration in the suspending medium of the chloroplasts in the dark induced accumulation of pyruvate in the stroma, similar to the case of light-driven active uptake. The effect was specific to Na^+ among various mono- and divalent cations. The apparent K_m for Na^+ was in the range 2–5 mM. The K_m for pyruvate was about 0.7 mM, which was similar to the value obtained in light-driven uptake. The possible role of Na^+ symport in active pyruvate uptake by C_4 mesophyll chloroplasts is discussed.

Pyruvate transport; Chloroplast membrane; Mesophyll chloroplast; C_4 photosynthesis; Na^+ jump; (*Panicum miliaceum*)

1. INTRODUCTION

The operation of the C_4 photosynthetic pathway requires extensive metabolite flow; intercellular flow between mesophyll and bundle sheath cells, and intracellular flow across the organelle membranes in each of the two cell types [1]. In this pathway, Pyr should be taken up into MCp to reproduce the primary CO_2 acceptor PEP. Recent findings [2–4] have demonstrated light-driven active uptake of Pyr into MCp of C_4 plants, maize and *Panicum miliaceum*. In a previous report [4] we showed a parallelism of the capacity of Pyr uptake and stromal alkalization and suggested that the energy source of the active Pyr uptake is not the stromal ATP but the pH gradient across the envelope. Testing the hypothesis of Pyr/H^+ symport, however, we have found that in the dark an artificial Na^+ gradient across the envelope drives pyruvate uptake, while the pH gradient itself gives negative results. An Na^+ gradient formed in the light may be the energy source for active Pyr uptake into C_4 MCp.

Correspondence address: R. Kanai, Department of Biochemistry, Faculty of Science, Saitama University, Urawa 338, Japan

Abbreviations: MCp, mesophyll chloroplasts; PEP, phospho*enol*pyruvate; Pyr, pyruvate; $[Pyr]_{in}$, pyruvate concentration in the sorbitol-impermeable space of chloroplasts; $[Pyr]_{ex}$, pyruvate concentration in the external medium

2. MATERIALS AND METHODS

Mesophyll protoplasts were isolated from young expanding leaves of *P. miliaceum* L., and intact MCp were purified therefrom according to [3] and suspended in either 50 mM Hepes-lysine (pH 7.6) and 0.35 M sorbitol ($-K^+$ medium) or 50 mM Hepes-KOH (pH 7.6) and 0.35 M sorbitol ($+K^+$ medium). Silicone oil filtering centrifugation was a modification of that in [3] and performed at 4°C on ice. In incubations for 7 s and longer, uptake was started by the addition of 50 μl MCp suspension to 150 μl suspending medium containing 0.2 mM [1-^{14}C]Pyr (free acid, 0.1 μCi per tube), [^{3}H]sorbitol (0.5 μCi per tube) and various concentrations of sodium gluconate partially substituting sorbitol. For shorter incubation times, the two-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

layer system of [5] was adopted: a 0.4 ml centrifuge tube contained, from the bottom: 20 μl of 1 M $HClO_4$, 70 μl silicone oil, 100 μl incubation mixture containing 10% (v/v) Percoll, [^{14}C]pyruvate, [^{3}H]sorbitol and sodium gluconate, 30 μl of the same silicone oil and 50 μl chloroplast suspension. On centrifugation the chloroplasts passed through the lower Percoll layer in 2 s on average [5], which is taken as the incubation time. Dark incubation was performed under a dim green fluorescent light and light incubation under a 300 W incandescent lamp (at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) (see [3,6] for other details of the method). Chl was determined as in [7].

3. RESULTS AND DISCUSSION

The MCp suspension was injected into media containing [1-^{14}C]Pyr and various metal ions and after 7 s incubation MCp were precipitated by centrifugation through the silicone oil layer. Among the cations tested, only Na^+ enhanced Pyr uptake over the control value (table 1). The same concentration of sodium gluconate gave essentially the same result. Inclusion of K^+ (about 25 mM) as a neutralizing ion in the suspending medium did not affect the result. In the routine experiments, however, $+K^+$ medium was used, because of some protective effect of K^+ from inactivation on storage (not shown).

Table 1

Pyruvate uptake induced by ion jump

Condition and addition	Pyruvate uptake (μmol·mg Chl^{-1}·h^{-1})	
	$-K^+$	$+K^+$
Light	4.3	5.0
Dark	1.2	0.6
+ 10 mM LiCl	1.3	0.5
NaCl	2.9	2.8
KCl	1.1	–
RbCl	0.6	0.3
CsCl	0.4	0.6
$MgCl_2$	1.0	0.5
$CaCl_2$	0.8	0.6
$MnCl_2$	0.8	0.9
Na-gluconate	2.1	2.8

The time course of Na^+-induced Pyr uptake is shown in fig.1. Both the initial uptake rate and the accumulation ratio ($[Pyr]_{in}/[Pyr]_{ex}$) increased with increasing Na^+ concentration. Pyr, once taken up into MCp, was retained after prolonged incubation up to 5 min. The initial uptake rates of Na^+-induced Pyr uptake measured in 7 s incubations were plotted vs Na^+ concentrations (fig.2). The result shows apparent saturation kinetics and a double-reciprocal plot (inset) gave a straight line except for the higher concentration region where the points deviated downward. The K_m for Na^+ was 4.8 mM in this experiment and in the range 2–5 mM in 3 separate experiments. This Na^+-induced capacity of Pyr uptake (initial uptake rate and accumulation ratio after 1 min incubation) decayed after the Na^+ jump with a half-life of about 2 min (table 2). Standing for 5 min after the jump almost nullified the Na^+ effect.

The above results show that an Na^+ jump in the dark drives Pyr uptake by MCp of *P. miliaceum*, and that the driving force, possibly an Na^+ gradient across the envelope, decays with a time course similar to that of the light-induced capacity of Pyr uptake (cf. figs 2,3 of [4]). This leads to the question as to whether the Na^+ gradient across the envelope is also the driving force of light-driven

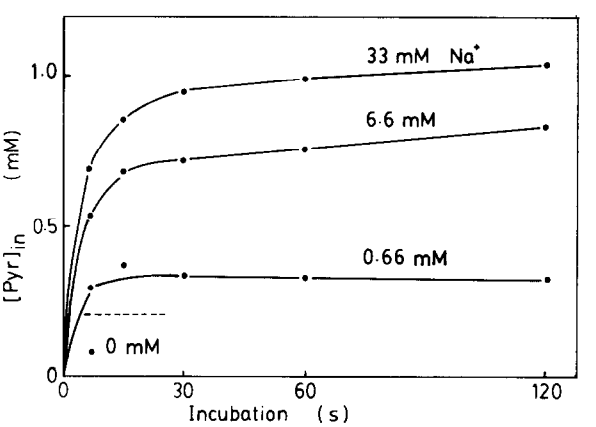

Fig.1. Time courses of Pyr uptake induced by an Na^+ jump. Incubation was started by the addition of MC to media containing sodium gluconate (final concentration as indicated), [1-^{14}C]Pyr and [^{3}H]sorbitol and terminated by centrifugation in a Microfuge (Beckman) at 10000 rpm for 30 s. Pyr taken up into the sorbitol-impermeable space was determined as in [7]. Dashed line denotes $[Pyr]_{ex}$.

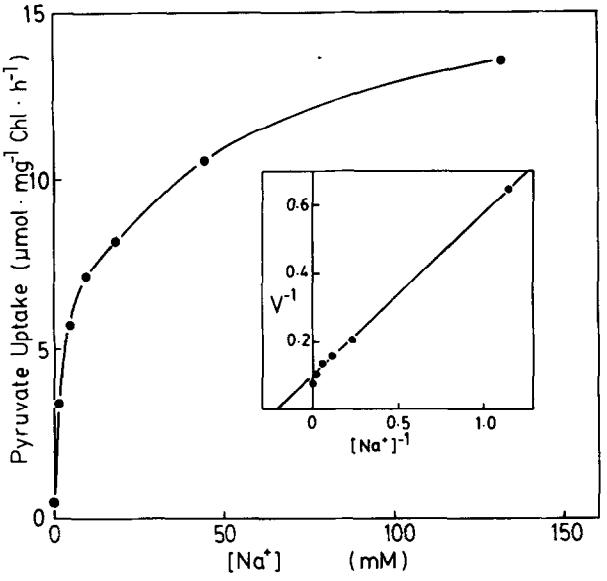

Fig.2. Na^+ concentration dependency of the initial (7 s) rate of Pyr uptake induced by an Na^+ jump. Incubation was as described in the legend to fig.1. (Inset) Double-reciprocal plot of the results.

Pyr uptake. Comparing the two cases, light uptake leads to up to 30-fold accumulation of Pyr in 2–5 min incubation [3], while the Na^+ jump induces less than 5-fold accumulation (fig.1). Besides, the light effect was additive to that of the Na^+ jump and the Na^+ jump further increased uptake in the light (table 3). These two observations may not support the idea that an Na^+ gradient is the sole energy source of Pyr uptake. However, the

Table 2

Decay of Pyr uptake capacity induced by an Na^+ jump

Time (min) after Na^+ jump	Initial uptake (μmol·mg^{-1} Chl·h^{-1})	Accumulation ratio ($[Pyr]_{in}/[Pyr]_{ex}$ after 1 min incubation)
0	5.6	2.9
1	3.7	1.5
5	1.3	1.1
Control (=0 mM Na^+)	0.6	0.95

Pyr was added at the indicated times after the Na^+ jump (8.75 mM)

Table 3

Effect of light on Pyr uptake induced by an Na^+ jump

Condition	Pyruvate uptake (μmol·mg^{-1} Chl·h^{-1})	
	Control	Na^+ jump ($[Na^+]$ = 131 mM)
Dark	1.0	16.2
Light	9.2	48.7

result shown in fig.3 shows that the K_m for Pyr in Na^+-induced uptake (0.68 mM) is quite similar to that in light-driven uptake (0.74 mM), suggesting that the same translocator is operating in both cases.

As stated in section 1, the Pyr uptake capacity of MCp was independent of the ATP level in the stroma but shows a good correlation with stromal pH [4]. However, an artificial pH gradient across the envelope induced by a pH jump of the medium could not drive Pyr uptake (unpublished). Therefore, the remaining candidate for the energy source of light-driven active Pyr uptake would be an ion gradient whose formation is tightly coupled with the pH gradient across the envelope and/or stromal alkalinization. If an Na^+ gradient is also the energy source for light-driven uptake, a possible mechanism of Pyr uptake in the light would be

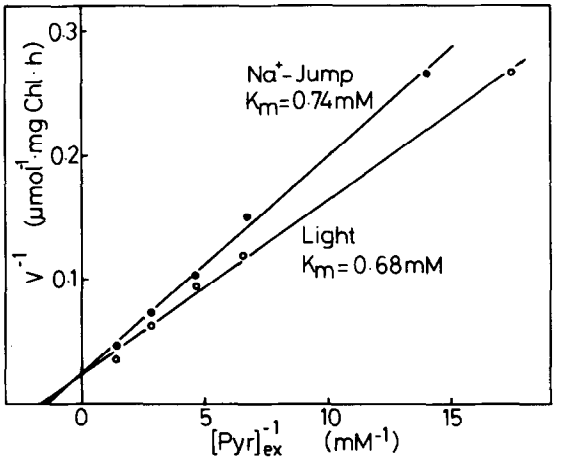

Fig.3. Double-reciprocal plots of the initial (2 s) rate of Pyr uptake induced by light and an Na^+ jump (8.75 mM). Incubation was as described in section 2 using the two-layer system of [6].

as follows: (i) light induces alkalinization of the stroma due to the proton pumping into the thylakoid lumen; (ii) a pH gradient (or proton-motive force) across the envelope forms an Na^+ gradient possibly through Na^+/H^+ antiport as reported for C_3 chloroplasts [8] and other systems [9]; (iii) Pyr is taken up by an Na^+/Pyr symport mechanism. In support of mechanism (ii), we found that an Na^+ jump induces a gradual increase in stromal pH (unpublished).

To test the above hypothesis, the exact concentration gradient of Na^+ across the envelope should be known. In C_3 chloroplasts, the stromal Na^+ concentration was reported to be 23 mM both in the light and dark [10] and Mg^{2+} stimulated Na^+ (or K^+)/H^+ exchange across the envelope [8]. Further work is now underway to determine the changes in Na^+ concentration in C_4 MCp during dark to light transition and an Na^+ jump as well as a quantitative correlation of Pyr and Na^+ uptake on pyruvate addition.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and by a grant from the Ministry of Agriculture, Forestry and Fisheries (GEP62-II-1-x).

REFERENCES

[1] Hatch, M.D. and Osmond, C.B. (1976) in: Encyclopedia of Plant Physiology (Stocking, C.R. and Heber, U. eds) vol.2, pp.144–184, Springer, Berlin.
[2] Flügge, U.I., Stitt, M. and Heldt, H.W. (1985) FEBS Lett. 183, 335–339.
[3] Ohnishi, J. and Kanai, R. (1987) Plant Cell Physiol. 28, 1–10.
[4] Ohnishi, J. and Kanai, R. (1987) Plant Cell Physiol. 28, 243–251.
[5] Howitz, K.T. and McCarty, R.E. (1985) Biochemistry 24, 2645–2652.
[6] Heldt, H.W. (1980) Methods Enzymol. 69, 604–613.
[7] Wintermans, J.F.G.M. and De Mots, A. (1965) Biochim. Biophys. Acta 109, 448–453.
[8] Huber, S.C. and Maury, W. (1980) Plant Physiol. 65, 350–354.
[9] Boron, W.F. (1983) J. Membrane Biol. 72, 1–16.
[10] Gimmler, H., Schäfer, G. and Heber, U. (1974) in: Proceedings of the Third International Congress on Photosynthesis (Avron, M. ed.) vol.3, pp.1381–1392, Elsevier, Amsterdam, New York.